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Note

The separation of some tricyclic antidepressants by high-performance liquid chromatography

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In recent years there has been much interest in the separation of drugs by high-performance liquid chromatography (HPLC). This technique is now being established in pharmaceutical analysis as a rapid qualitative and quantitative procedure and as an alternative to gas chromatography.

It is rather surprising that so little work has been done on the HPLC separation of tricyclic antidepressants. A thorough study was reported by Knox and Jurand[1] who compared two chromatographic methods based respectively on ion-pair partition and basic alumina adsorption chromatography for a large series of psychosedative drugs including some of the tricyclic antidepressants, amitriptyline (AMI), nortriptyline (NOR), protriptyline (PRO), imipramine (IMI) and trimipramine (TRI). Watson and Stewart[2] reported a separation on a silica gel column of only the three dibenzocycloheptanes (AMI, NOR and PRO). A methanol–ammonia solvent was used.

Here we report the separation and the chromatographic behaviour of some more of these substances, particularly those containing an aminoalkane side chain. The compounds are eluted from a micro-silica gel column with a much weaker solvent mixture than the one used by Watson and Stewart. Special attention has been paid to the amount of base added to the eluent which, as expected, has been found to have a great influence on the separation time and the capacity factor, k', and in this way on the resolution.

EXPERIMENTAL

The investigated antidepressant drugs are tabulated in scheme I. The pure drug substances were obtained from Specia*, MSD*, Geigy-Ciba** and Labaz*. The compounds were used as their hydrochloride salts, except trimipramine maleate, and injected as 0.1% solutions in dichloromethane. Volumes of 1 μl of these solutions were applied to the column.

The Varian Model LC 8500 chromatograph used was fitted with an ultraviolet detector operating at a fixed wavelength of 254 nm. A Mikropak Si-10 column

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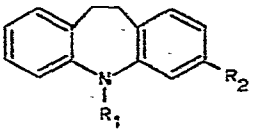
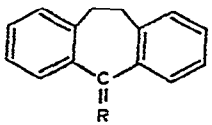
A. Dihydrodibenzoazepines



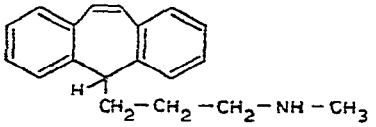
R_1	R_2	
$CH_2-CH_2-CH_2-N(CH_3)_2$	H	Imipramine (IMI)
$CH_2-CH_2-CH_2-N(CH_3)_2$	Cl	Chlorimipramine (ClIMI)
$CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	H	Trimipramine (TRI)
$CH_2-CH_2-CH_2-NH-CH_3$	H	Desipramine (DESI)

B. Dibenzocycloheptanes



R	
$=CH-CH_2-CH_2-N(CH_3)_2$	Amitriptyline (AMI)
$=CH-CH_2-CH_2-NHCH_3$	Nortriptyline (NOR)

C. Dibenzocycloheptene



Protriptyline (PRO)

Scheme I

(25 cm × 2 mm I.D.), particle size, 10 μm, was chosen. All of the experiments were carried out at ambient temperature. The eluting solvent was dichloromethane–*n*-heptane (1:1) to which 0.2% isopropanol and different amounts of *n*-propylamine were added (preferred amount, 0.13%). A flow-rate of 30 ml/h was adopted ($\Delta p \approx 30$ atm).

RESULTS AND DISCUSSION

Silica was chosen as the adsorbent because its slightly acidic properties allow a good retention of the basic drugs. The mixture dichloromethane–*n*-heptane was used as a solvent with an intermediate strength according to Saunders[3]. Isopropanol was added as a modifier in order to achieve a uniform activity of the silica. This improves the reproducibility.

Two groups of substances were easily identified in all of the chromatograms as shown in Fig. 1. The substances which contained in their side chain the more polar secondary amine function (NOR, DESI and PRO) interacted more strongly with the

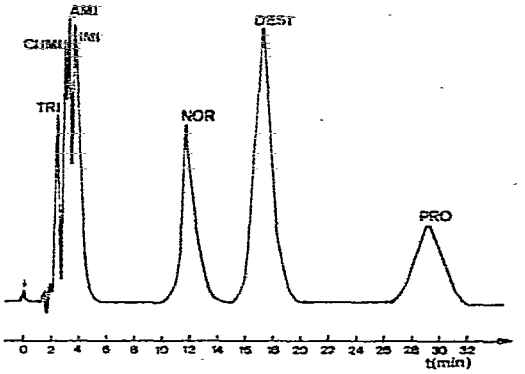


Fig. 1. General elution pattern of the seven antidepressant drug substances. Eluent: dichloromethane–*n*-heptane (1:1), 0.2% isopropanol, 0.25% propylamine. Flow-rate, 30 ml/h.

stationary phase than those containing a tertiary amine function (TRI, CIMI, AMI and IMI).

The most important factor influencing the chromatographic migration is the base content (propylamine) of the eluent. In neutral eluents, basic compounds frequently give rise to tailing because of chemisorption on the silica. Preliminary experiments showed the necessity of adding a base to the eluent in order to obtain an accelerated and better elution. This effect is illustrated in Fig. 2, and in Fig. 3 the speed of the chromatographic process, as given by the capacity factor k' of some of the components, is plotted as a function of the propylamine content of the eluent.

The concentration of propylamine was optimized using the UNIPLEX procedure described by King and Deming[4]. This consists in a formal strategy for finding rapidly an optimal value for a criterion. It is not necessary to describe the method here in detail, and readers are referred to the original literature. UNIPLEX is a special (and simple) case of the SIMPLEX optimization procedures, the application of which for chromatographic purposes has been described by several workers[5,6]. In the present investigation, we used an optimization criterion incorporating the peak to valley ratio as described by Morgan and Deming[5]. The resulting optimal separation of the four tertiary amine compounds is given in Fig. 4.

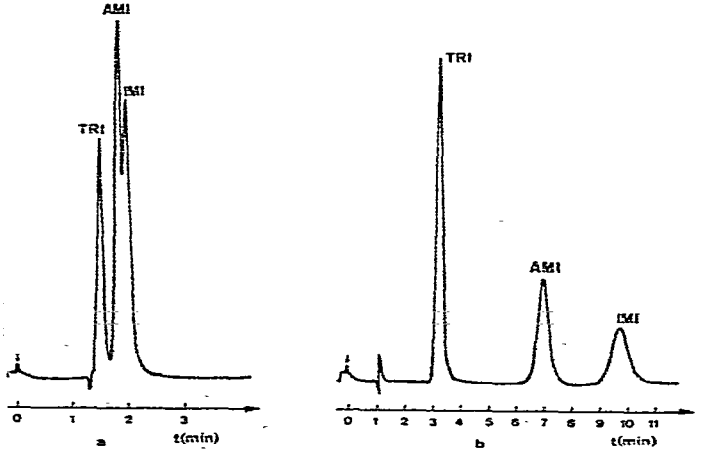


Fig. 2. Influence of the propylamine content of the eluent. Conditions as in Fig. 1 except the propylamine concentrations were 0.35% (a) and 0.05% (b).

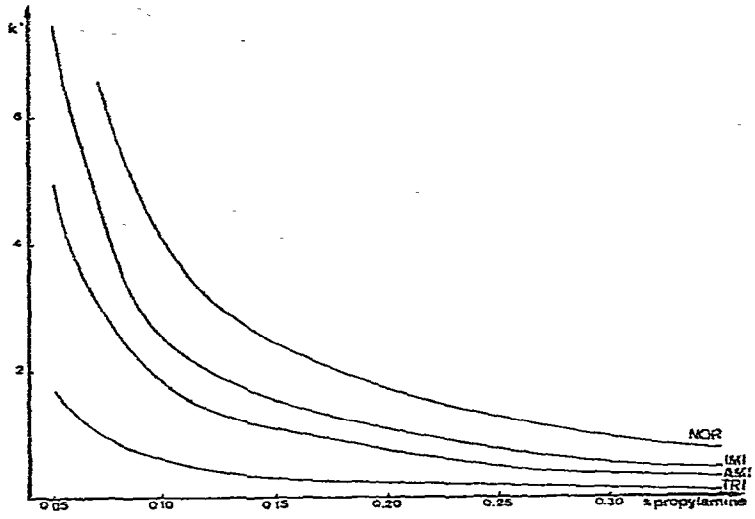


Fig. 3. Influence of the propylamine concentration on the rate of the chromatographic migration of some components as given by their capacity factor k'. The k' scale for NOR has been expanded five times.

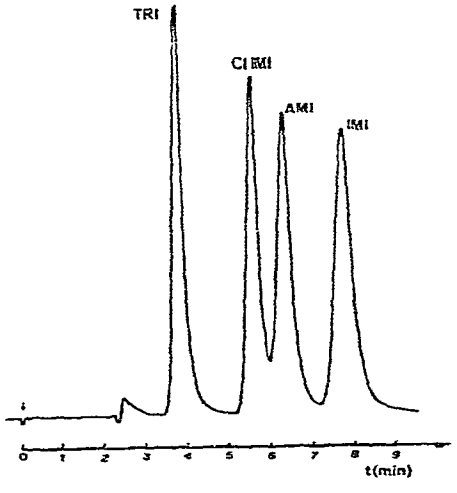


Fig. 4. Optimal separation of the four tertiary amine compounds (TRI, ClIMI, AMI and IMI) on Mikropak Si-10. Eluent: dichloromethane–*n*-heptane (1:1), 0.2% isopropanol, 0.13% *n*-propylamine. Flow-rate, 30 ml/h.

ACKNOWLEDGEMENT

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